

### **In the Specification:**

Please amend the specification as shown:

Please delete the following paragraphs listed from page 102, line 18, to page 104, line 14, and replace with the following paragraphs:

**Preparation and stimulation of splenocytes for cytokine production.** Spleens are harvested from the various groups of mice (n=2-3) and pooled in p60 petri dishes containing about 4ml RPMI-10 media (RPMI-1640, 10% fetal bovine serum, 50 µg/ml gentamycin). All steps in splenocyte preparations and stimulations are done aseptically. Spleens are minced with curved scissors into fine pieces and then drawn through a 5 cc syringe attached to an 18G needle several times to thoroughly resuspend cells. Cells are then expelled through a nylon mesh strainer into a 50 ml polypropylene tube. Cells are washed with RPMI-10, red blood cells were lysed with ACK lysis buffer (Sigma, St. Louis, MO), and washed 3 more times with RPMI-10. Cells were then counted by trypan blue exclusion, and resuspended in RPMI-10 containing 80 U/ml rat IL-2 (Sigma, St. Louis, MO) to a final cell concentration of  $2 \times 10^7$  cells/ml. Cells to be used for intracellular cytokine staining are stimulated in 96-well flat-bottom plates (Becton Dickenson Labware, Lincoln Park, NJ), and cells to be used for cytokine analysis of bulk culture supernatants are stimulated in 96-well U-bottom plates (Becton Dickenson Labware, Lincoln Park, NJ). One hundred microliters of cells are dispensed into wells of a 96-well tissue culture plate for a final concentration of  $2 \times 10^6$  cells/well. Stimulations are conducted by adding 100 µl of the appropriate peptide or inactivated influenza virus diluted in RPMI-10. CD8<sup>+</sup> T cells are stimulated with either the K<sup>d</sup>-restricted HA<sub>533-541</sub> peptide (IYSTVASSL) (Winter, Fields, and Brownlee, 1981) (SEQ ID NO: 5) or the K<sup>d</sup>-restricted NP<sub>147-155</sub> peptide (TYQRTRALV) Rotzchke *et al.*, 1990) (SEQ ID NO: 6). CD4<sup>+</sup> T cells are stimulated with inactivated influenza virus (13,000 HAU per well of boiled influenza virus plus 13,000 HAU per well of formalin-inactivated influenza virus) plus anti-CD28 (1 µg/ml) and anti-CD49d (1 µg/ml) (Waldrop *et al.*, 1998). Negative control stimulations are done with media alone. Cells are then incubated as described below to detect extracellular cytokines by ELISA or intracellular cytokines by FACS staining.

**Chromium release assay for CTL.** CTL responses to influenza HA and NP are measured using procedures well known to those in the art (see, *e.g.*, Current Protocols In Immunology, John E. Coligan *et al.* (eds), Unit 3, Wiley and Sons, New York, NY 1994, and yearly updates including

2002). The synthetic peptide HA<sub>533-541</sub> IYSTVASSL (Winter, Fields, and Brownlee, 1981) (**SEQ ID NO: 5**) or NP<sub>147-155</sub> TYQRTRALV (Rotzschke *et al.*, 1990) (**SEQ ID NO: 6**) are used as the peptide in the target preparation step. Responder splenocytes from each animal are washed with RPMI-10 and resuspended to a final concentration of  $6.3 \times 10^6$  cells/ml in RPMI-10 containing 10 U/ml rat IL-2 (Sigma, St. Louis, MO). Stimulator splenocytes are prepared from naïve, syngeneic mice and suspended in RPMI-10 at a concentration of  $1 \times 10^7$  cells/ml. Mitomycin C is added to a final concentration of 25 µg/ml. Cells are incubated at 37°C/5%CO<sub>2</sub> for 30 minutes and then washed 3 times with RPMI-10. The stimulator cells are then resuspended to a concentration of  $2.4 \times 10^6$  cells/ml and pulsed with HA peptide at a final concentration of  $9 \times 10^{-6}$ M or with NP peptide at a final concentration of  $2 \times 10^{-6}$ M in RPMI-10 and 10 U/ml IL-2 for 2 hours at 37°C/5% CO<sub>2</sub>. The peptide-pulsed stimulator cells ( $2.4 \times 10^6$ ) and responder cells ( $6.3 \times 10^6$ ) are then co-incubated in 24-well plates in a volume of 2 ml SM media (RPMI-10, 1 mM non-essential amino acids, 1 mM sodium pyruvate) for 5 days at 37°C/5%CO<sub>2</sub>. A chromium-release assay is used to measure the ability of the *in vitro* stimulated responders (now called effectors) to lyse peptide-pulsed mouse mastocytoma P815 cells (MHC matched, H-2d). P815 cells are labeled with <sup>51</sup>Cr by taking 0.1 ml aliquots of p815 in RPMI-10 and adding 25 µl FBS and 0.1 mCi radiolabeled sodium chromate (NEN, Boston, MA) in 0.2 ml normal saline. Target cells are incubated for 2 hours at 37°C/5%CO<sub>2</sub>, washed 3 times with RPMI-10 and resuspended in 15 ml polypropylene tubes containing RPMI-10 plus HA ( $9 \times 10^{-6}$ M) or NP ( $1 \times 10^{-6}$ ) peptide. Targets are incubated for 2 hours at 37°C/5%CO<sub>2</sub>. The radiolabeled, peptide-pulsed targets are added to individual wells of a 96-well plate at  $5 \times 10^4$  cells per well in RPMI-10. Stimulated responder cells from individual immunization groups (now effector cells) are collected, washed 3 times with RPMI-10, and added to individual wells of the 96-well plate containing the target cells for a final volume of 0.2 ml/well. Effector to target ratios are 50:1, 25:1, 12.5:1 and 6.25:1. Cells are incubated for 5 hours at 37°C/5%CO<sub>2</sub> and cell lysis is measured by liquid scintillation counting of 25 µl aliquots of supernatants. Percent specific lysis of labeled target cells for a given effector cell sample is  $[100 \times (\text{Cr release in sample-spontaneous release sample}) / (\text{maximum Cr release-spontaneous release sample})]$ . Spontaneous chromium release is the amount of radioactive released from targets without the addition of effector cells. Maximum chromium release is the amount of radioactivity released following lysis of target cells after the addition of TritonX-100 to a final concentration of 1%. Spontaneous release should not exceed 15%.

Please delete the paragraph listed on page 106, line 1 to line 6 and replace with the following paragraph:

**Tetramers.** HA and NP tetramers may be used to quantitate HA- and NP-specific CD8<sup>+</sup> T cell responses following HA or NP immunization. Tetramers are prepared essentially as described previously (Flynn *et al.*, 1998). The present example utilizes the H-2K<sup>d</sup> MHC class I glycoprotein complexed the synthetic influenza A/PR/8/34 virus peptide HA<sub>533-541</sub> (IYSTVASSL) (Winter, Fields, and Brownlee, 1981) (SEQ ID NO: 5) or NP<sub>147-155</sub> (TYQRTRALV) (Rotzschke *et al.*, 1990) (SEQ ID NO: 6).